A Simple and Robust Method for the Determination of Total Mercury in Fish Tissue Using Continuous Flow – Cold Vapour ICP-MS and an External Standards Calibration

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A SIMPLE AND ROBUST METHOD FOR THE DETERMINATION OF TOTAL MERCURY IN FISH TISSUE USING CONTINUOUS FLOW - COLD VAPOUR ICP-MS AND AN EXTERNAL STANDARDS CALIBRATION

by

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ABSTRACT

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A simple and robust method for the determination of total mercury in fish tissue is presented. The method used a continuous flow of sample to the ICP-MS and external standards were used to calibrate the instrument, thus eliminating the need for spiking samples. The method is offered as an alternative to atomic absorption spectroscopy, and flow injection or isotope dilution ICP-MS methods for determining Hg in fish tissue. Accuracy of the method was determined by analyzing standard reference material for dogfish muscle and liver tissue (DORM-2 and DOLT-2). Our mean concentration for DORM-2 was 4.64 ± 0.36 μg g^{-1} compared to a certified value of 4.64 ± 0.26 μg/g. For DOLT-2 our mean value was 2.28 ± 0.48 μg g^{-1} compared to a certified value of 2.14 ± 0.28 μg g^{-1}. A comparison of data generated by this method and atomic absorption analyses found no difference in the relationship between fish length and Hg concentration in muscle tissue ($p>0.05$). Precision based on repeated analyses of various samples ranged from 2 to 20%. The method detection limit was determined to be 0.035 ng g^{-1}

RÉSUMÉ

Veinott, G. and Miller-Banoub, J. 2004. A simple and robust method for the determination of total mercury in fish tissue using continuous flow - cold vapour ICP-MS and an external standards calibration. Can Tech. Rep. Fish. Aquat. Sci. 2527: iv + 10 p.

Nous présentons ici une méthode simple et robuste pour doser le mercure total dans les tissus de poissons. La méthode fait appel au passage en continu de l'échantillon dans un spectromètre ICP-MS qui a été étalonné avec des échantillons externes, ce qui nous dispense de la nécessité d'utiliser des ajouts connus. Cette méthode remplace avantageusement le recours à la spectroscopie d'absorption atomique et aux méthodes ICP-MS par injection de flux ou dilution isotopique pour la mesure du Hg dans les tissus de poissons. Nous avons établi l'exactitude de la méthode en analysant des matériaux de référence pour les tissus musculaires et hépatiques d'aiguillat (DORM-2 et DOLT-2). Notre concentration moyenne pour DORM-2 était de 4,64 ± 0,36 μg·g^{-1} contre une valeur certifiée de 4,64 ± 0,26 μg·g^{-1}. Pour DOLT-2, notre valeur moyenne était de 2,28 ± 0,48 μg·g^{-1} contre une valeur certifiée de 2,14 ± 0,28 μg·g^{-1}. La comparaison des données obtenues par cette méthode et par absorption atomique n'a fait ressortir aucune différence dans la relation entre la longueur des poissons et la concentration de Hg dans le tissu musculaire ($p>0,05$). La précision obtenue après des analyses répétées de divers échantillons allait de 2 % à 20 %. La limite de détection de la méthode a été établie à 0,035 ng g^{-1}.

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INTRODUCTION

The inductively coupled plasma-mass spectrometer (ICP-MS) has the rapid multielement capabilities of ICP-optical emission spectrometry (OES) and superior detection limits to graphite furnace atomic absorption spectrometry (GFAAS). This combination of assets has produced a powerful instrument for quantifying much of the periodic table especially the higher mass elements (Z>100) where fewer interferences occur. ICP-MS, therefore, is ideally suited for the determination of Hg, which has a high mass and is often in low concentrations. Unfortunately, because of memory effects, resulting in long washout times (e.g. Yoshinaga and Morita 1997), the analysis of Hg by solution ICP-MS has not been favoured.

Successful determination of Hg using cold vapour ICP-MS techniques have been reported (Hintlemann and Ogrinc 2002; Chiou *et al.* 2001; Christopher *et al.* 2001; Shith 1993). However, despite the reported success, fisheries researchers have appeared not to have adopted this method for Hg analyses. A search of publications in the field of aquatic sciences for the years 2002/2003 did not produce a single reference that used cold vapour ICP-MS for Hg analyses. Thomas (2001) suggested that the major reason that ICP-MS has failed to out pace ICP-OES and AAS as the instrument of choice for routine multielement analyses was not the higher price, but the perception that ICP-MS was a complex "research" instrument. The resistance to adopt ICP-MS as a Hg detector may be the same. Thomas (2001) also suggested that ICP-MS researches have not done a very good job of communicating the benefits and ease of operation of a modern ICP-MS.

The purpose of this paper, therefore, is to present a simple and robust method for the determination of Hg in fish tissue by ICP-MS. No modifications were made to the ICP-MS, except to replace the spray chamber with the manufacturer's adapter for direct introduction of gasses. The manufacturer's liquid-gas separator was used, as is, and reagents were purchased from common suppliers. This method is based on the work of Hintelmann and Ogrine (2002) but is an alternative to flow injection and isotope dilution ICP-MS methods as well as the more common AAS method for Hg analysis of fish tissue. Some advantages of this method include: using the same digestion procedure for multielement solution ICP-MS analyses and cold vapour Hg analysis, which eliminates the need for a separate digestion for Hg; using disposable polypropylene tubes for sample dilution, storage, and analysis; using external standards to calibrate the instrument, thus eliminating the need for spiking the samples; and using a continuous flow of sample so that a stable Hg signal is obtained and auto sampling is available.

METHODS

REAGENTS

Concentrated Omnitrace® nitric acid and trace clean hydrochloric acid were used without further purification. A 0.2N bromine monochloride solution (BrCl) was produced by dissolving 1.08 g of reagent grade potassium bromide in 100 ml of concentrated HCl in a glass bottle and stirring for 1 h. Next 1.52 g of reagent grade potassium bromate was slowly added to the solution while continuing to stir. Care must be taken to prevent exposure to toxic fumes released at this

time. The bottle was then loosely capped and stirred for an additional hour. The final solution must be stored at room temperature and covered in aluminium foil to prevent contamination from ambient Hg. A 20% m/v solution of hydroxylamine hydrochloride ($NH_2OH*HCl$) was made in 100 ml of ultrapure (18 MΣ) water. The glass bottle was tightly capped and stored in a plastic bag. A 0.05% (m/v) solution of sodium borohydride ($NaBH_4$) was prepared in a 0.05% (m/v) solution of sodium hydroxide (NaOH).

Calibration standards were prepared by diluting a 100 $\mu g\ g^{-1}$ Hg stock solution. The dilutions were carried out in a series of steps to reduce measurement error. The 100 $\mu g\ g^{-1}$ solution was diluted to 1 $\mu g\ g^{-1}$ then the 1 $\mu g\ g^{-1}$ solution was diluted to 100 $ng\ g^{-1}$ which was diluted to 1, 2, 5, and 10 $ng\ g^{-1}$ solutions in 5% (v/v) nitric acid, as needed.

SAMPLE DIGESTION

Samples consisted of the National Research Council of Canada reference materials DORM-2 (dogfish muscle) and DOLT-2 (dogfish liver), and skeletal muscle from lake trout (*Salvelinus namaycush*). Samples of frozen lake trout were lyophilized for 48 hours then homogenized using an agate mortar and pestle. Approximately 0.1 g of dried tissue was digested in 5 ml of nitric acid in a closed vessel microwave digester. The pressure in the digestion vessel was raised to 100 psi held there for 30 min then increased to 140 psi for 5 min. Samples were allowed to cool before transfering to 50 ml polypropylene centrifuge tubes and diluting to 40 ml with ultrapure water. Approximately 0.1 g of DORM-2 and DOLT-2 were digested in the same manner. Blanks consisting of 0.5 ml of H_2O were also treated to the same process.

To oxidize all the Hg present to Hg^{2+}, between 3 and 5 ml of BrCl was added to the dilute samples. The presence of a yellow colour in the samples after 12 hours indicated that excess BrCl was present. To neutralize the excess BrCl 1 ml of $NH_2OH*HCl$ was added to each sample. After neutralizing the samples the solution was brought to a final volume of 50ml.

INSTRUMENTATION

A Perkin Elmer/Sciex Elan 6100 ICP-MS was used to detect Hg in the samples. The spray chamber was removed and replaced by an adapter that allowed for the direct introduction of gasses to the ICP. Mercury was liberated from the sample by mixing the sample with the $Na(BH_4)$ reductant in a Perkin Elmer Mercury/Hydride chemifold (Fig. 1). The sample and reductant were fed continuously into the chemifold by a peristaltic pump at a rate of approximately 1.25 ml/min. Between samples, the sample line was rinsed with a 2% (v/v) nitric acid solution. ICP-MS operating parameters are given in Table 1. The ICP-MS was calibrated by analyzing 5 standard solutions with nominal Hg concentrations of 0, 1, 2, 5, and 10 $ng\ g^{-1}$.

RESULTS AND DISCUSSION

CALIBRATION

By continually pumping sample and reductant into the chemifold a relatively stable Hg signal was obtained (Fig. 2). The stable signal eliminates the problems of transient signals from flow injection techniques, and allows for the simple calculation of a mean intensity and its standard deviation. The relative standard deviation of the signal intensity for a 1ng g^{-1} Hg solution was generally less than 10%.

A steady output from the ICP-MS also lends itself to a calibration strategy that employs external standards. By using external standards there is no need to spike samples, which is time consuming and can be a source of error and contamination. Typical r^2 values for calibration curves in this study were between 0.999 and 0.99999.

ACCURACY AND PRECISION

Accuracy of the calibration was determined by analyzing the National Research Council of Canada certified materials DORM-2 and DOLT-2. Over a two month period our mean Hg concentration in DORM-2 (n = 12) was 4.64 ± 0.36 μg g^{-1} whereas the certified value is 4.64 ± 0.26 μg g^{-1}. For DOLT-2 (n = 4) our mean value was 2.28 ± 0.48 μg g^{-1} compared to a certified value of 2.14 ± 0.28 μg g^{-1}. Our ± values are 3x the standard error of the mean, and represent relative errors of 8 and 21% respectively.

We also compared our results on lake trout tissue to AAS results produced by the Université du Québec à Montréal (Fig 3). At Hg concentrations above 0.5 :g g^{-1} the results from the two methods diverged. However, the differences between the two methods were not constant, suggesting random error. A regression analyses comparing mercury concentration in muscle tissue to fork length (Fig 4) revealed no difference between the two methods. The slope and elevation of the two regression lines were not significantly different from each other (t-test $p>0.05$).

To estimate the precision of the method, replicate analyses of skeletal muscle from lake trout were performed. The range of relative standard deviations for the replicate analyses was from 3 to 20% (Table 2).

INSTRUMENT AND SAMPLE STABILITY

Stability of the instrument was monitored by repeatedly analyzing 3 solutions (High Hg, Low Hg, and 0 Hg) every 30 min for 7 h. The ICP-MS was calibrated with fresh standard solutions then set to cycle through the 3 samples. Figure 5 demonstrates that there was no significant drift of the signal for low Hg or 0 Hg, however, High Hg began to drift after 4 hours. There was an approximate 5% decline in apparent concentration, but the decline was not linear.

Depending on the application, large batches of samples (>30) may require recalibration after 4 hours, which can be automated with the instrument's software.

To evaluate the long term stability of the sample solutions, 8 samples were analyzed on 4 different days over a 7 day period. Samples were kept in the same 50 ml plastic tubes that they were originally transferred to after digestion. Sample concentration means and relative standard deviations are listed in Table 3. There was no systematic increase or decrease in concentration during the 7 days (Fig. 6) and the variability, as indicated by the RSDs (Table 3), suggests a smaller change in concentration between days than that expected from replicate analyses of the same material. The data suggest that samples can be stored for up to a week without compromise, and therefore, can be held until a batch is ready for analysis, which reduces analytical costs.

SENSITIVITY AND DETECTION LIMITS

Sensitivity, defined as counts per second (cps) ng^{-1} g^{-1} Hg varied from day to day and has been shown to be dependent on nebulizer flow rate, forward power and ion lens voltage[7]. Sensitivity was also dependent on pump speed. However, maximizing sensitivity did not translate into optimal performance. It was noted that at high pump speeds and nebulizer gas flow rates of greater than 1 L min^{-1} the introduction of the sample and the reductant into the chemifold caused splashing which affected the stability of the Hg signal. Therefore, the conditions listed in table 1 were decided upon for optimal sensitivity and signal stability. Under these operating conditions the sensitivity of the method was on average 2×10^5 cps ng^{-1} g^{-1} Hg.

It should also be noted that with our instrument the torch ignition sequence automatically reduces the nebulizer gas flow to zero just prior to torch ignition. After a plasma has been achieved the nebulizer flow is increased to the set value. At this point in the ignition sequence any sample or reductant in the chemifold is blown into the liquid/gas separator chamber causing splashing and reduced signal stability. To eliminate this problem, the cap for the liquid/gas separator chamber was not put in place until after the nebulizer flow was at its set value. It was also noted that the filter in the liquid gas separator was not necessary under these operating conditions and was therefore removed.

One definition of the detection limit of a method is that concentration which produces a signal that is significantly greater ($p<0.05$) than the signal produced by a sample with no analyte present. Based on the mean and standard error of our method blanks (n=10) the detection limit for this method was 0.035 ng/g. Given the sample sizes and dilution factors used in this study the concentration of Hg in wet fish tissue necessary for detection (assuming 80% moisture) would be 3.5 ng g^{-1}. This value is within the range of detection limits reported for other ICP-MS mercury methods (Christopher *et al.* 2001, Karunasagar *et al.* 1998, Woller *et al.* 1997, Yoshinaga and Morita 1997, Smith 1993), and greater than two orders of magnitude less than the accepted safe consumption concentration in fish of 500 ng g^{-1}.

Our method, therefore, is more than adequate for routine analyses of total Hg in fish tissue and has applications in studies examining the effect of flooding on Hg in fish, effect of

size of fish on Hg concentrations, food web accumulation, etc. As well, although we focused on fish tissue there is no obvious practical or theoretical reason why this method would not work on other types of biota. However, the method does not have the precision necessary for Hg isotope tracer studies or the detection limit for research on pristine waters where Hg concentrations are on the order of a few pg g^{-1}. Improvements in precision and sensitivity could be achieved by conducting the sample preparation in a class 100 clean room or by using purer grade chemicals. However, for many applications the simplicity of our approach may be more attractive than the additional cost or time commitment required to attain improved precision or sensitivity.

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The authors would like to acknowledge Dr. Holger Hintelmann and Brian Dimock from Trent University for their work on this subject, and thank them for assisting us and answering our many questions regarding mercury analysis by ICP-MS. Also, thanks to Dr. Marc Lucotte and Isabelle Rheault from the Université du Québec à Montréal for allowing us use of their data. Funding for this project was provided by the Canadian Collaborative Mercury Research Network (COMERN) and Fisheries and Oceans Canada.

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Table1. ICP-MS operating parameters.

Parameter	Value
Nebulizer gas	0.98 L min^{-1}
RF Power	1000 Watts
Mode	Peak Hop
Dual Detector	On
Auto Lens	On
Sweeps/Reading	10
Readings/Replicate	1
Replicates/Analysis	25
Dwell Time	100 msec
Sample Flush	120 sec
Read Delay	60 sec
Wash	300 sec

Table 2. Estimate of precision of Hg determinations in fish tissue using the ICP-MS method. Concentrations are μg g^{-1} wet weight.

Sample	Mean	Std Dev	RSD (%)	n
MR-125	0.50	0.08	16	3
MR-146	0.21	0.01	3	2
MR-160	0.32	0.01	4	3
MR-202	0.59	0.12	20	2

Table 3. Precision of long term sample stability test. Concentrations are μg g^{-1} dry weight.

Sample	Mean	Std Dev	RSD %	n
Dolt-2	2.007	0.09	4.66	4
Dorm-2-a	4.339	0.18	4.14	4
Dorm-2-b	4.402	0.08	1.80	4
MR-207	1.061	0.05	4.27	4
MR-222	0.886	0.07	7.39	4
MR-Pike201	3.218	0.22	6.70	4
MR-W5	0.295	0.03	11.16	4

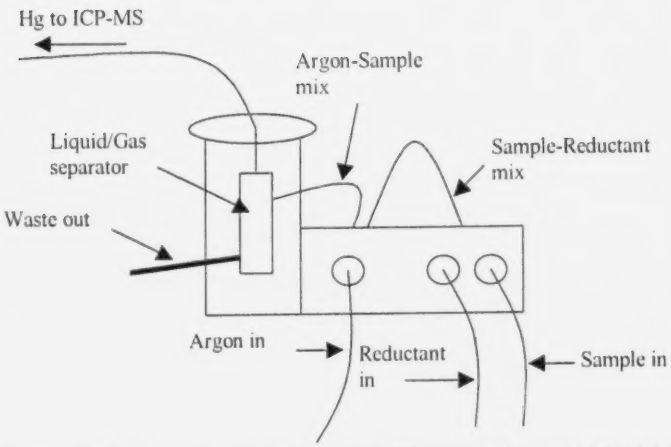

Figure 1. Schematic of the Perkin-Elymer Mercury/Hydride chemifold

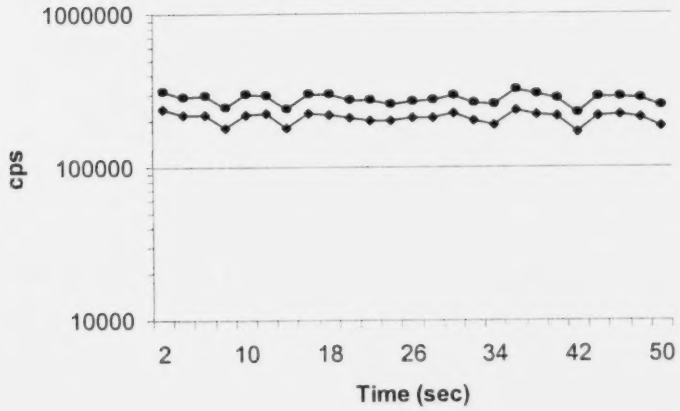

Figure 2. Profile of a typical 1ng/g Hg signal from the ICP-MS. Circles represent Hg^{202}, diamonds represent Hg^{200}.

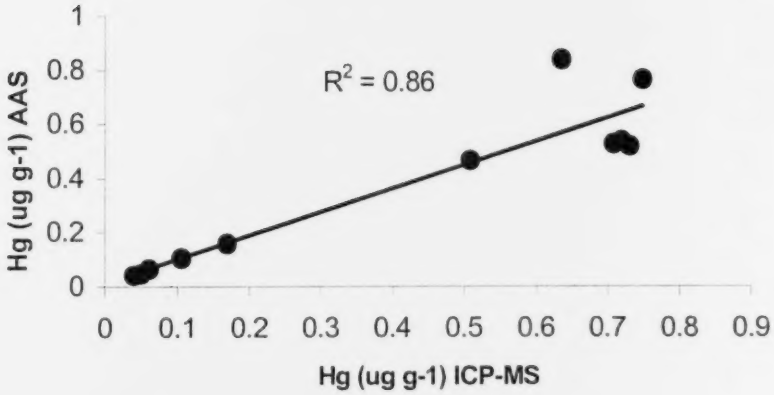

Figure 3. Concentration of Hg (wet weight) in lake trout muscle tissue by ICP-MS and AAS

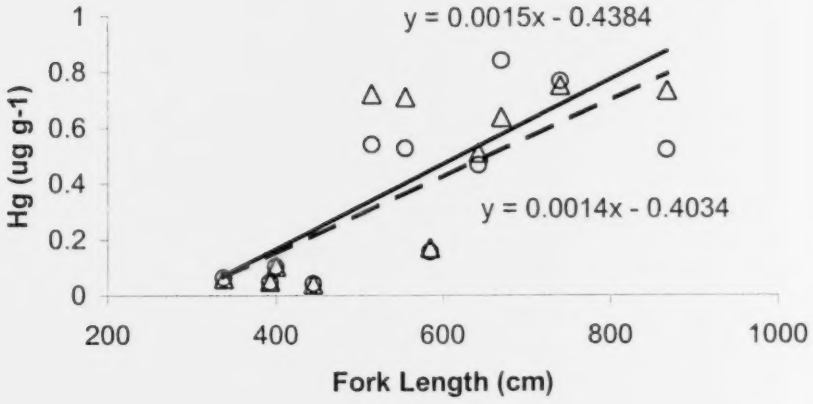

Figure 4. Comparison of regression analyses of fork length and Hg concentration (wet weight) in lake trout using ICP-MS and AAS data. Solid line and triangles represent ICP-MS data and broken line and circles represent AAS data.

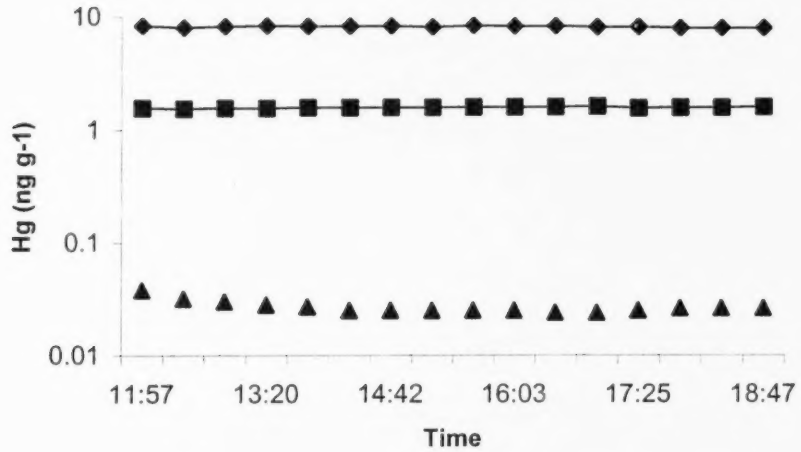

Figure 5. Seven hour instrument stability test. Each symbol represents repeated analysis of the same solution. Diamonds represent High Hg, squares represent Low Hg, and triangles represent 0 Hg.

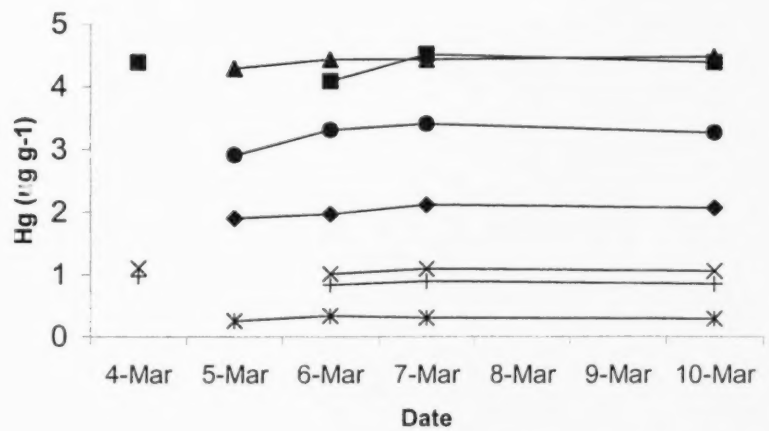

Figure 6. Stability test of sample solutions. Each symbol represents a sample solution that was analyzed on different days. Concentrations are reported on the basis of dry tissue weight.